NON-MENDELIAN MEGAGAMETOGENESIS IN ARABIDOPSIS[1,2]

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ANY interference with the independent assortment of genes generally affects male and female gametes equally, as is the case in the "quasi linkage" or affinity phenomenon of the house mouse (MICHIE and WALLACE 1953). Lack of independence may be limited, however, to the female gametophyte, as demonstrated by LONGLEY (1945). This "false linkage" is brought about by the similar orientation of marked, knob-bearing chromosomes.

Unequal representation of alleles may result by nonrandom polarization of the bivalents during meiosis of the female sporocyte. Preferential segregation (STURTEVANT 1934) is generally not expected to have genetic consequences for the sperms or microspores (exceptions exist, however—cf. SMITH-WHITE 1948). The mechanism and consequences of preferential disjunction—neocentromeric activity induced by chromosome knobs and unequal marker recovery—have been demonstrated in maize by RHOADES (1942, 1952) and his associates.

Selective elimination of certain chromosomes during spermatogenesis may also result in selective segregation (cf. SMITH-STOCKING 1936) or in meiotic drive (SANDLER and NOVITSKI 1957).

Abnormal segregation is based relatively rarely on meiotic anomalies. Postmeiotic selection is a more common cause of deviations from the Mendelian expectations. The female gametes are less frequently affected by this selection than the male gametophytes. As early as 1902, CORRENS demonstrated that the abnormal phenotypic ratios observed were caused by selective fertilization, leaving the validity of the Mendelian laws unimpaired. This mechanism was called *certatio* by NILSSON (1915). Selective fertilization is common both in animals (WHITING 1935) and in plants. Several male gametophyte factors have been analyzed extensively in corn (JONES 1924; BRINK 1925; EMERSON 1934; BRIEGER, TIDBURY and TSENG 1939; SCHWARTZ 1950; LONGLEY 1961; and others).

Complete elimination of the male gamete is also common and is being studied by many workers because agronomic importance is involved (cf. NIELSEN 1962). Collapse of the female apparatus without higher degree of male sterility has also been observed (STROMAN 1941; DAS 1957; CASADY, HEYNE and WEIBEL 1960).

RENNER's (1921) observations on early postmeiotic selection of the functional megaspore (Gonenkonkurrenz, megaspore competition) have not been confirmed

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outside the Oenotheras. Similar megaspore selection should be common, however, among various plant species (cf. SCHNARF 1929).

The following analysis is concerned with an Arabidopsis mutation which is not transmitted through the egg, is inherited relatively normally through the sperm, produces abnormal ratios of linked markers, and appears itself to segregate in a non-Mendelian way. It also affects recombination. These three unusual consequences of the mutation (lack of "female transmission", "preferential segregation", and differential recombination in the two gametophytes) are determined by a single pleiotropic factor.

MATERIALS AND METHODS

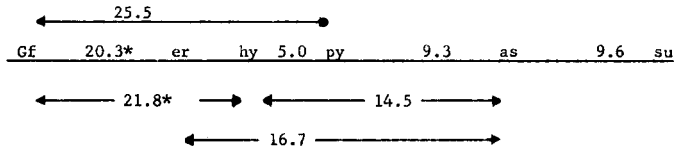
The mutant (*Gf*) was found in an X-irradiated population of our Columbia wild type (12,000r to 24-hour presoaked seed). It was recognized only because the same single-seed progeny contained another linked mutant *py*[1], a conditional lethal, requiring 2,5-dimethyl-4-amino-pyrimidine for survival (RÉDEI 1960). Owing to the presence of *Gf* in repulsion, the pyrimidine requiring class appeared in excess. Curiously, in the same single-seed progeny we found, in addition, an independent pollen-abortion factor (RÉDEI 1964) and also an albina type.

Linkage information was obtained, unless otherwise stated, from F_2 in coupling phase. Linkage intensities were calculated according to the product method (IMMER 1930). Symbols, and brief descriptions of relevant genes, are: *Gf*, female gametophyte factor; *er*, compact erecta type; *hy*, high hypocotyl; *py*, pyrimidine requiring; *as*, asymmetric rosette leaves; *su*, sulfurata, bright yellow-green. These markers belong to the second linkage group of Arabidopsis, established in our laboratory (Table 1).

Embryological samples were fixed in ALLEN's B-15 with evacuation, embedded in Tissuemat,

TABLE 1

Partial map of linkage group 2 with the recombination values observed in F_2 (repulsion information only)



	Gf	*er*	*hy*	*py*	*as*	*su*
Gf	....	....	....	20.7 ± 1.3 (988)†	....	....
er	....	....	nil (15,395)	7.0 ± 0.4 (5108)	16.7 ± 0.5 (7073)	24.0 ± 0.8 (4465)
hy	....	....	....	5.0 ± 0.3 (6295)	14.5 ± 0.1 (15126)	....
py	....	....	....	....	9.3 ± 0.4 (6696)	....
as	....	....	....	....	....	9.6 ± 0.8 (4465)

Map distance from *Gf* presented here only for the male. Numbers in parenthesis indicate the size of the respective populations.
* Indirectly estimated.
† Combined value from testcrosses (male and female).

sectioned to 6 to 8 microns, stained in HEIDENHAIN's iron hematoxylin, and differentiated with picric acid. Cytological samples were processed according to STEINITZ-SEARS's technique (1963).

RESULTS

In the second generation from an X-rayed seed, a ratio of 9/16 py^+ and 7/16 py was observed, which indicated abnormality. Wild-phenotype plants of these abnormally segregating populations were subjected to progeny tests. The majority of them appeared normal, however, and less than one third repeated the abnormal phenotypic ratio. About one sixth appeared wild type; some of these, however, regularly produced a few percent py. Those few plants (ca. 2%) which threw only a few py were arbitrarily classified as wild type, since if only a very small mutant class is expected, misclassification can not be avoided in small populations (Table 2). It was very easy to identify the abnormal families, since the phenotypic ratio of $py^+ : py$ resembled a backcross more than an F_2. Despite the fact that py is a seedling lethal unless thiamine or its pyrimidine moiety is supplied, there was a small but consistent excess of the mutant class. The homogeneity test was positive, with a very high probability of 0.80. In normal sib families or outcrosses, the py marker segregated in remarkably good agreement with the Mendelian expectation (Table 3).

The observed abnormal ratio suggested that the transmission of the py^+ strand

TABLE 2

Progeny-test of the selfed wild-phenotype plants from abnormal families (Gf py+/Gf+ py)

	Number of progenies obtained			
Generation	Abnormal $\frac{Gf\ py^+}{Gf^+\ py}$	Normal $\frac{Gf^+\ py^+}{Gf^+\ py}$	Wild* $\frac{Gf^+\ py^+}{Gf^+\ py^+}$ or $\frac{Gf\ py^+}{Gf^+\ py^+}$	Sum
X_3	2	3	1	6
X_4	11	23	6	40
X_5	54	93	26	173
Total number	67	119	33	219
Observed percent	30.6%	54.3%	15.1%	100%
Expected with 32% male transmission of *Gf*	30.2%	53.4%	14.6%	100%

* Included also is 2.1% of $Gf\ py/Gf^+\ py^+$ constitution, which in small populations may not be distinguished from the the wild type.

TABLE 3

Segregation of Gf+ py × Gf py+ *and* Gf+ py × Gf+ py+ *families* (F_2)

Type and number of families	Observed marker		Ratio			Degrees of freedom
	py^+	py	py/py^+	χ^2	P	
Abnormal 67	5726	5968	0.96	55.3	0.80	66
Normal 160	12923	4290	0.33	0.0544	0.80	1

is very poor. The pollen of 11 abnormal heterozygotes (carriers of *Gf*) was examined, and pollen abortion was not observed.

Since the seeds are arranged in linear order on the replum, sterility can easily be estimated by holding the ripe fruits against the light. The fertility of Arabidopsis is greatly influenced by environmental factors. Seed-set is reduced if the temperature rises slightly above the optimal level. Silique fertility was determined in families segregating for *py*. The seed-set even in the normal families differed considerably in the various experiments. It was obvious, however, that the fertility of the abnormal families was reduced and that the difference was primarily genetically determined (Table 4).

Germination tests failed to show much difference between normal and abnormal families. Apparently the germination of the abnormals was even slightly higher. This indicates that the seed-set count reflects correctly the absolute differences in mortality (Table 5).

It was obvious that we were dealing with an ovule-abortion or egg-lethality factor. Tests were carried out to detect the transmission of this ovule-abortion factor. Four plants which were identified through early seed-set as carriers of

TABLE 4

Silique fertility of normal and abnormal families

Experiment No.	Type of families	No. of plants	No. of fruits examined	Seed set			Fertility
				Total	Average per fruit	Percent	
1	Normal	20	108	4651	43.1	100.0	94.3
	Abnormal	11	68	1889	27.8	64.5	75.6
2	Normal	22	67	1860	27.8	100.0	76.1
	Abnormal	15	50	892	17.8	64.0	53.2
3	Normal	17	53	1431	27.0	100.0	82.5
	Abnormal	11	43	695	16.2	60.0	55.4
4	Normal	1	20	599	30.0	100.0	68.5
	Abnormal	1	20	384	19.2	64.0	50.9
Total	Normal	60	248	8541	34.4	100.0	...
	Abnormal	38	181	3860	21.3	61.9	...

TABLE 5

Germination of normal and abnormal families from seed samples of 200 each

Type and number of families tested	Degrees of freedom	Total number of seeds	Germinated			Germination		Confidence limits (P = .02)
			Wild	Mutant	Total	Percent	Mean	
Normal 22	35	7200	4642	1639	6281	87.24	174.47	2.85
Abnormal 8	20	4200	1827	1967	3794	90.33	180.66	4.07
						Difference →	6.19	...
						Sum ⟶	...	6.92

Gf (genotype *Gf py*$^+$/*Gf*$^+$ *py*) were crossed as a female parent to normal males which were genetically marked with *as*, in the second linkage group. The transmission of the *Gf* factor is detectable in the second-generation progeny by the excess of the *as* marker. Altogether 386 chromosomes were tested, and none of them carried *Gf*. Nearly five times more *py* alleles came through than *py*$^+$. The *py* families segregated in a ratio of about 1:2:1, while the *py*$^+$ progenies gave a reasonably good fit to a 3:1 ratio. Retests of those *py*$^+$ families which exhibited the largest deviation from 3:1 showed that the deviation was due to random sampling. Female transmission of the *Gf* factor was not observed in the experiment, and the probability of 1% transmission is as low as 0.001 (Table 6).

If a gene is not transmitted through the egg, and there is no meiotic or post-meiotic selection, only 50% viable seed is expected. Experimentally, about 62% was found. This is a highly significant deviation from expectation (Tables 4, 7). It is obvious that some kind of selection is responsible for the modification of the haploid segregation ratio.

Male transmission of *Gf* is also reduced, although no difference was detectable by staining of pollen. There was considerable variation among plants in the male transmission of *Gf*. This can be attributed to physiological differences among flowers or anthers. The ten plants tested were genetically identical. In compe-

TABLE 6

Number of chromosomes transmitted through the female in crosses of $\frac{+\,\text{py}\,+}{\text{Gf}\,+\,+}$ *female* $\times$ $\frac{+\,+\,\text{as}}{+\,+\,\text{as}}$ *male*

py	*py*$^+$		
320	66		
	↓ Segregation of the *py*$^+$ families		
	as$^+$	*as*	Ratio
Observed	6413	2269	2.83:1

TABLE 7

Comparison of the number of good seeds per silique per plant in the abnormal families, and half of that number in the normal families

Experiment No.	Total number good seeds per fruit of abnormals*	Half the number of good seeds per fruit of normals*	Difference		Probability
			No. of seeds	Expressed as percentage of the normal	
1	27.89	21.58	6.31	22.6%	0.0002
2	17.36	13.74	3.62	26.3%	0.0005
3	16.45	13.39	3.06	22.9%	0.0200
4	19.20	15.00	4.20	28.0%	0.0002

* Discrepancies from values in Table 4 are due to the fact that here the number of seed per fruit was calculated on a per-plant basis.

tition with the pollen carrying the normal strand, the abnormal sperms were successful in one third of the cases (Table 8).

It is easy to grow and classify large populations of Arabidopsis but it is cumbersome to make testcrosses, especially with stocks of lower viability. The test of possible gametophytic differences can be carried out, however, only in backcrosses.

The estimation of linkage intensity on the female side is relatively simple. Since *Gf* itself has no transmission, the frequency of markers linked in coupling to it is equal to the recombination frequency. On the male side, all four products of recombination can be recovered. For the estimation of linkage, however, only one of the parental and one of the crossover strands have been considered, in order to avoid complications due to differential transmission. Since two generations are needed to get the complete testcross estimates, considerable labor is involved in obtaining information about all strands. From the cross *py as* × *Gf* +/+ *py*, the *py* class was not subjected to further progeny-test. From the number of the *py* plants, the recombination fraction obtained in the female was subtracted, and in this way the number of Gf^+ *py* parental strands has been estimated. From among the crossover strands, only the number of Gf^+ py^+ has been used. If recombination in male and female is identical, we can obtain in this way direct information of linkage intensity. Since the data demonstrate differential recombination in the two sexes (lower in the female) the estimate obtained for the male is a minimal figure, and the actual recombination value is somewhat higher. In spite of the fact that a minimal estimate has been used for the male,

TABLE 8

Male transmission of Gf *in crosses of*

$$\frac{+\,+\,\text{as}}{+\,+\,\text{as}} \textit{ or } \frac{+\,\text{py as}}{+\,\text{py as}} \textit{ female} \times \frac{\text{Gf}\,+\,+}{+\,+\,+} \textit{ male}$$

Experiment No.	No. of chromosomes transmitted		Transmission in percent
	Gf+	*Gf*	
1	14	5	26.3
2	25	8	24.2
3	12	10	45.5
4	21	15	41.7
5	31	13	29.5
6	96	42	30.4
7	54	20	27.0
8	25	8	24.2
9	33	14	29.8
10	43	31	41.9
Total	354	166	31.9
Segregation of the *as* marker indicating the transmission of *Gf*			
	as+	*as*	Ratio
Gf present	10,572	7,604	1.4:1
Gf absent	14,564	4,573	3.2:1

a significant difference in recombination was found between megaspores and microspores (Table 9).

Additional linkage studies have been carried out in F_2 populations involving several markers. For the region left of *py*, corrections have been made for crossing-over values, while to the right of *py*, recombination values were used. Differential recombination for male and female has been considered only for the region which separated *Gf* from the nearest marker involved in a particular cross. Though the F_2 generation does not give complete information on all the strands it allows some insight into recombination. It is obvious that the presence of *Gf* modifies the phenotypic frequencies. The deviation is greater if the linkage is tighter. This is especially clear in the F_2 from Gf^+ *er as su* × *Gf* er^+ as^+ su^+, where 54% of the total population is homozygous for the proximal marker *er*, while only 39% is homozygous for the distant marker *su* (Table 10). It should be mentioned, however, that the transmission or the survival of *su* is somewhat lower in any case than that of *er*. In the two other crosses involving *hy as* or *py as* strands, where the transmission is fairly normal regularly, in the abnormal populations the homozygous recessive-marker class has a frequency of 51% in the

TABLE 9

Differential recombination in female and male between Gf *and* py *factors*

Gametes	Total tested	Number of recombinants	Recombination fraction	Degrees of freedom	P
Male	475	121	25.5* ± 2.7	9	...
Female	513	82	16.0 ± 1.0	9	...
Difference	...	...	9.5 ± 2.9	13	0.01

* Minimal estimate.

TABLE 10

Linkage studies with Gf

Gf–20.3–*er*–16.7–*as*–9.6–*su*
10.1*

	Phenotype frequencies								
	Parental		Single recombinant types				Double recombinants		
	+ + +	er as su	er as +	+ + su	+ as su	er + +	er + su	+ as +	Total
+ *er as su* × + + + +									
Observed no.	2799	621	179	170	247	391	8	50	4465
	62.7%	13.9%	4.0%	3.8%	5.5%	8.8%	0.2%	1.1%	100.0%
+ *er as su* × *Gf* + + +									
Observed no.	685	559	146	72	91	286	7	5	1851
	37.0%	30.2%	7.9%	3.9%	4.9%	15.5%	0.4%	0.3%	100.1%
Expected with 50% ♂ transmission of *Gf*	37.4%	30.4%	6.8%	2.8%	4.9%	15.1%	1.4%	1.3%	100.1%

* Estimated crossing-over frequency in the female.

case of *hy* and 53% in the case of *py*. The corresponding percentage for the more distal marker *as* is 39 in the former, and 43 in the latter, population (Tables 11 and 12). No frequency should exceed 25% in the absence of *Gf*, and the observations are in reasonable agreement with this expectation in normal populations (Tables 10, 11, 12).

Though "crossing over is one of the most variable phenomena" (GOWEN 1919), and so is genetic transmission (cf. Table 8), the observed phenotype frequencies (a function of these two variables) are in reasonable agreement with the expec-

TABLE 11

Linkage studies with Gf

Gf–21.8–*hy*–14.5–*as* 11.6*					
	Phenotype frequencies				
	Parental		Recombinants		
	+ +	*hy as*	*hy*	*as*	Total
+ *hy as* × + + +					
Observed no.	10,426	2,704	928	1,068	15,126
	68.9%	17.9%	6.1%	7.1%	100.0%
+ *hy as* × *Gf* + +					
Observed no.	2,479	1,882	939	263	5,563
	44.6%	33.8%	16.9%	4.7%	100.0%
Expected with 50% ♂ transmission of *Gf*	42.8%	37.4%	14.1%	5.6%	99.9%
+ *hy* + *as* × *Gf* + *py* +					
Observed no.	505	401	184	38	1,128
	44.7%	35.5%	16.3%	3.4%	99.9%

* Estimated crossing-over frequency in female.

TABLE 12

Linkage studies with Gf

Gf–25.5–*py*–9.3–*as* 16.0*					
	Phenotype frequencies				
	Parental		Recombinants		
	+ +	*py as*	*py*	*as*	Total
+ *py as* × + + +					
Observed no.	4,726	1,376	298	296	6,696
	70.6%	20.5%	4.5%	4.4%	100.0%
+ *py as* × *Gf* + +					
Observed no.	1,009	899	347	114	2,369
	42.6%	38.0%	14.7%	4.8%	100.1%
Expected with 32% ♂ transmission of *Gf*	44.2%	42.9%	9.3%	3.6%	100.0%

* Recombination value in female.

tations based on data collected in separate experiments. Though the F_2 data are not suitable for far-reaching conclusions, it appears that *Gf* may modify crossing-over frequencies in several ways. In the first abnormal population (Table 10) the double crossovers between regions II and III are slightly decreased. Data of Tables 11 and 12, however, indicate higher recombination than expected between *hy* and *as* and also between *py* and *as*, in the three populations tested. Further studies are needed to clarify the situation.

To detect the biological basis of the abnormalities caused by *Gf*, ovules were sectioned from 40 plants, wild types from the F_2 of a $(Gf + py +) \times (+ hy + as)$ hybrid, about half of which were expected to be abnormal. Progeny-tests indicated that only 17 (42.5%) were abnormal. The embryo sacs appeared normal (Figure 1a,b,c). In one case, however, perfectly well developed, 8-nucleate, twin embryo sacs (Figure 2b) were observed, and in another instance two-nucleate twin embryo sacs were found (Figure 2a). Twin megasporocytes were displayed by certain ovules (Figures 3 a,b,c; 4 a,b). Often several ovules in a single fruit showed this anomaly (Figure 3b). Arabidopsis regularly develops an archesporium with several cells, of which only one normally grows into a sporocyte (VENDENDRIES 1909). MISRA (1962) also found only one functional archespore.

Under normal conditions, all the ovules in an ovary are more or less synchronized in development. Occasionally deviations from this rule have been observed; one ovule was in the tetrad stage and its neighbor in the eight-nucleate stage (Figure 1a). Asynchronous meiosis in the twin megasporocytes has also been seen (Figure 3c).

Cytological studies were carried out to a limited extent. All meioses seen both in mega- and microsporocytes appeared normal.

DISCUSSION

The failure of female transmission of *Gf* could be interpreted as the result of a gene determining egg-lethality. Hereditary differences affecting the haplophase are known in various organisms, e.g. male-gametophyte factors of Zea (JONES 1924), Matthiola (KAPPERT 1937), Oenothera (RENNER 1919), Melandrium, (CORRENS 1921), and Arabidopsis (RÉDEI 1964). The occurrence of female-gametophyte factors is much less common. SINGLETON (1932) reported briefly on an unusual "lethal ovule" (*lo*) factor on chromosome 4 of maize, which apparently was not transmitted through the egg, while its male transmission was not seriously impaired. No biological mechanism was suggested. The stock which contained *lo* is probably lost (personal communication of DR. M. G. NEUFFER). There are some other reports on embryo abortion caused by one recessive (STROMAN 1941), or a few dominant genes (CASADY, HEYNE, and WEIBEL 1960). Aneuploidy may also cause disturbances of this nature (SHEPHERD 1960).

The *Gf* mutant of Arabidopsis seems to be unique. If it is a simple megagametophyte lethal (lethal since no transmission), the maximum seed-set in its

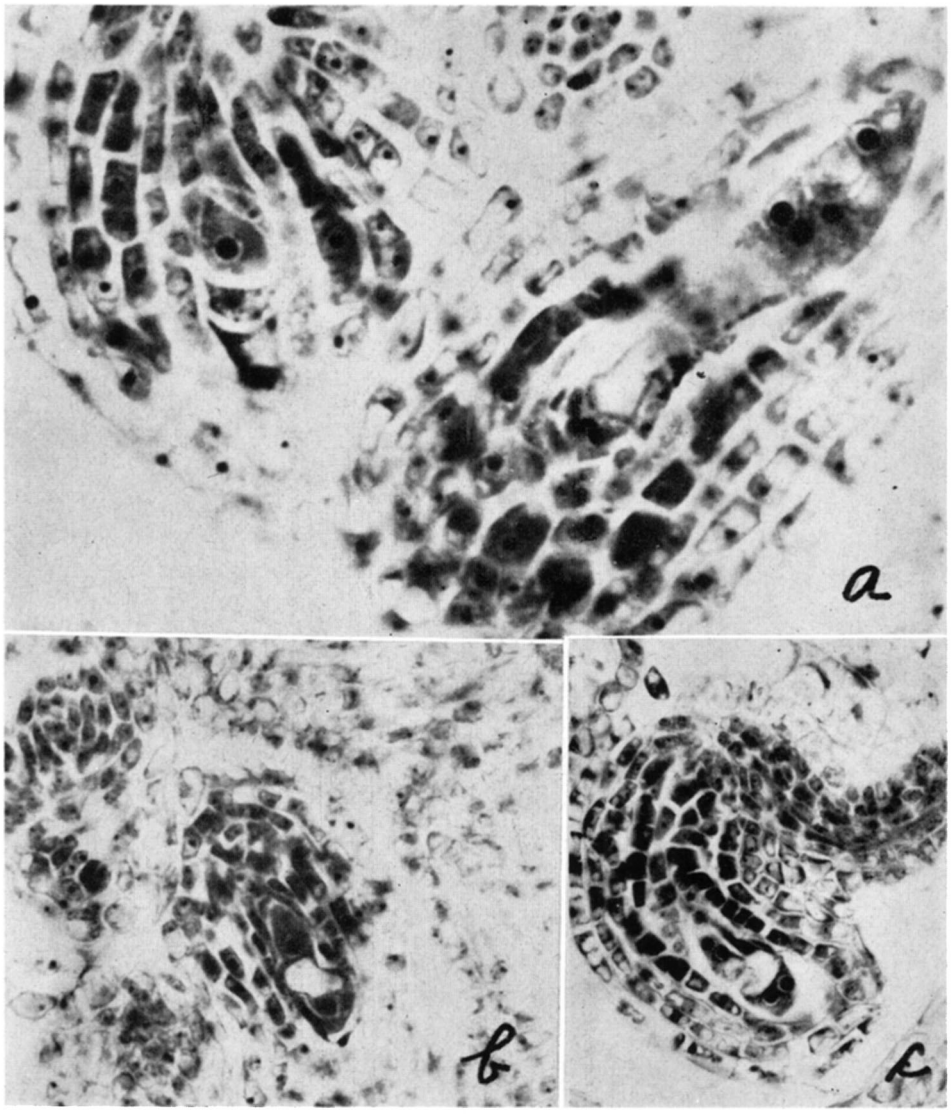


FIG. 1—a. (Left) Late tetrad in one ovule, the chalazal spore is functional, the other three are in the process of disintegration. (Right) Eight-nucleate stage of the embryo sac. In the lower corner only two antipodals are visible, in the middle the two polar nuclei are close; just above them is the egg. Of the two stretched pear-shaped synergids, only one contains a nucleus; the other nucleus remained in the next section. Note the unusual asynchronous development of the two neighbor ovules. Otherwise both the tetrad and the embryo sac are completely normal, polygonum type. b. Normal embryo sac at the two-nucleate stage. c. Normal embryo sac at the four-nucleate stage.

presence would be expected to be 50%. Since the seed-set is definitely higher ($P = 0.001$), it appears that the megaspores are produced in a non-Mendelian way.

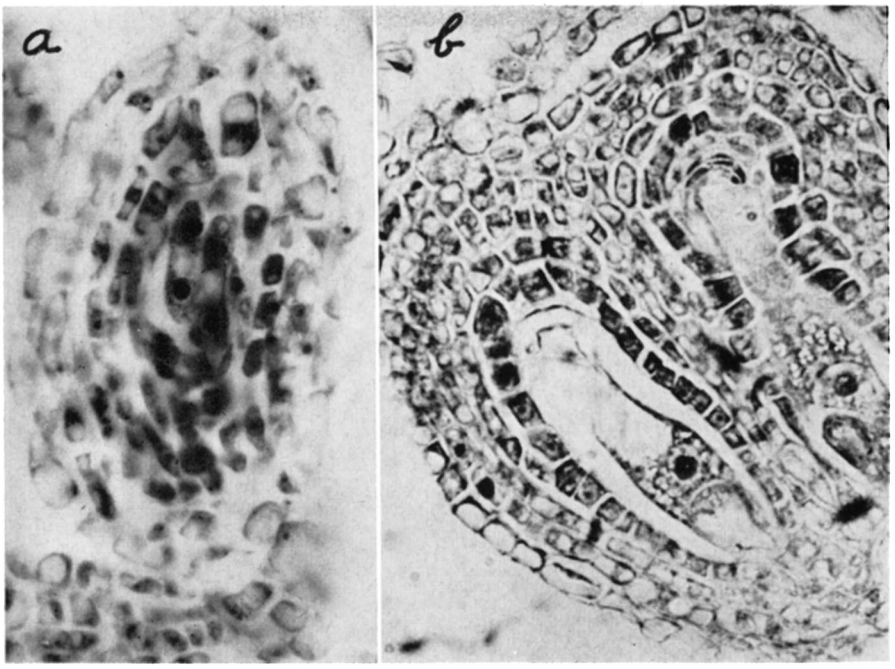


FIG. 2.—Unusual ovules, a. With two identical two-nucleate embryo sacs. b. With twin embryo sacs at the eight-nucleate stage. In the lower embryo sac the fused polar nuclei, in the upper embryo sac the egg nucleus is visible. Note the difference between the two twin embryo sacs: *a* developed from two neighboring archespores. *b* probably arose from two sporocytes which were originally separated by at least one cell. (Compare this with the structure of the leftmost ovule in Figure 3b).

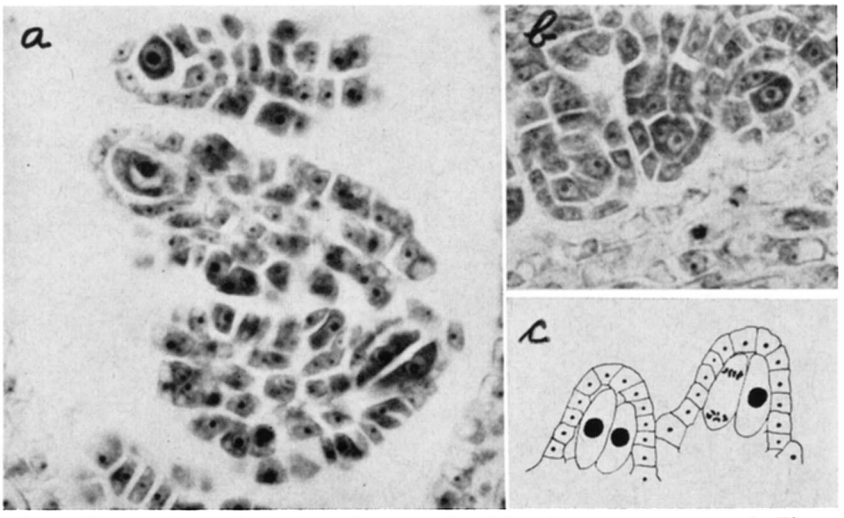


FIG. 3.—a. Two ovules with single and one ovule with twin megasporocytes. b. Three young ovules in each two archespores are distinguished by their larger nuclei. c. Two twin megasporocytes, in one precocious meiosis. (This drawing was made from an acetocarmine preparation.)

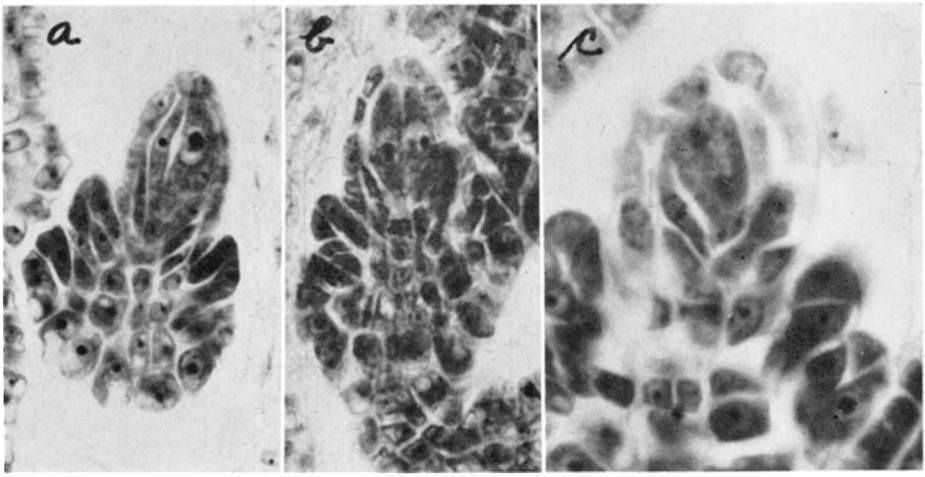


FIG. 4.—a. One of the twin sporocytes lagging in development. b. Identical sized twin megaspore mother cells just before meiosis. c. Normal single megasporocyte at the end of the first meiotic division.

Deviation from Mendelian frequencies may be brought about by various mechanisms: (i) preferential segregation (STURTEVANT 1934; RHOADES 1942; CATCHESIDE 1944; SHULT, DESBOROUGH, and LINDEGREN 1962); (ii) selection in a multisporic embryo sac (cf. MAHESHWARI 1950; or (iii) megaspore competition (RENNER 1921). There is no cytological indication in Arabidopsis for preferential segregation. Since Arabidopsis chromosomes are extremely small (3.7–1.1 μ, STEINITZ-SEARS 1963), and heteromorphism could hardly be detected unless it would double the size of a chromosome arm, this mechanism cannot be ruled out. There was no evidence for neocentric activity; however, the appearance of neocentromeres is not a necessary prerequisite of preferential segregation. If cytological evidence on preferential polarization is not available, genetic information alone may lead to controversy even in cases where tetrad analysis is feasible (MATHIESON 1956).

If two or more genetically dissimilar spores cooperate in embro sac development, selective elimination of certain types may interfere with Mendelian ratios. Arabidopsis, however, is definitely monosporic.

In the Oenotheras generally, the micropylar megaspore functions. If this cell is of an unfavorable genetic constitution, the chalazal or another cell may take over and become functional (RENNER-effect). Such a mechanism may not be limited to Oenothera. SCHNARF (1929) lists many species where it is not absolutely predetermined which cell of the megaspore tetrads will be functional. In the Arabidopsis material studied, there was no embryological evidence for megaspore competition (Figure 5).

In several species the development of multiple megasporocytes is regular, and this is the rule in several close relatives of Arabidopsis. In *Cardamine pratensis* and *Sisymbrium taraxacifolium*, from among about a dozen archespores, many

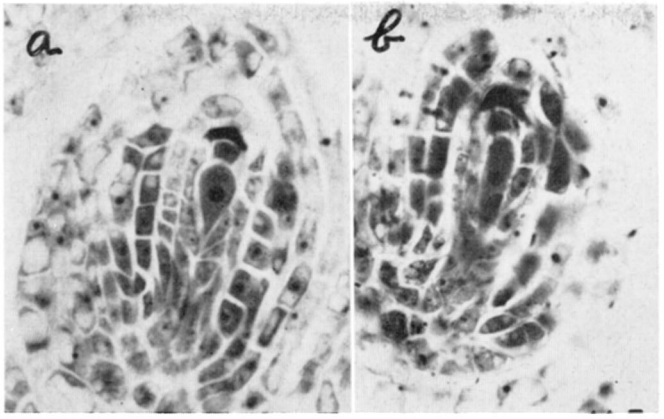


FIG. 5.—a. Normal megaspore tetrad formation. It is clear that the chalazal cell is the functional one. b. A tetrad which by superficial inspection may indicate megaspore competition. However, the basal small spore (note the clearly different nucleus from that of the nucellar cells) is in a different plane, and belongs probably to another tetrad which is in the process of degeneration because of the basal orientation of the gametophyte factor.

develop into sporocytes, but only a single functional megaspore is produced. *Sisymbrium officinale* displays five or six enlarged archespores, of which only one gives rise to a tetrad (VENDENDRIES 1909). VENDENDRIES also studied Arabidopsis (synonym *Sisymbrium thalianum*), where he observed several small archespores but only a single sporocyte. The formation of multiple sporocytes provides opportunities for postmeiotic selection. Such selection has not, to our knowledge, been studied genetically in the Cruciferae.

The abnormal segregation of the megaspores and the occurrence of twin primary megasporocytes (2n) is probably not coincidental in this material. If twin megasporocyte dyads are produced, selection may favor that basal secondary sporocyte (n), which is free from *Gf* because of the fortunate orientation of the bivalent concerned in meiosis I. There is another opportunity for selection between basal megaspores of reversely oriented twin tetrads. Both mechanisms should increase the seed-set over 50% in a case when one of the homologous chromosomes has no transmission. In a plant which carries *Gf*, and therefore displays 62% relative seed-set, twin sporocytes would be expected in at least 48% of the ovules. Half of them however might not be detected as twins because of the inappropriate plane of sectioning. Since only 42.5% of the plants sampled were of the proper genotype about 10% of the ovules are expected to display double sporocytes. Biased sampling should modify this minimal estimate.

Quantitative information on the occurrence of twinning has not been collected, and statistical analysis is not feasible. The observed frequency of twinning has been quite low, but generally if one ovule in an ovary contained twins, the same phenomenon was also detected in its several sisters (cf. Figure 3b,c). The frequency of twin sporocytes appeared higher in younger ovules and twinning was apparently less frequently observed during or after meiosis.

A similar selection has been suggested by KAPPERT (1935) to explain the deficiency of the recessive class in crosses of Linum strains differing in flower color.

The relation of the partial female-sterility to differential recombination in the two gametophytes is not understood. Selection alone should not have any effect on recombination frequency unless crossing over between *Gf* and the centromere interferes with another exchange between *Gf* and *py*. Suppression of recombination in the heterogametic sex is widespread in animals. Differential recombination in the two gametophytes for certain chromosomal regions is well documented in a few cases in plants (STADLER 1926; RHOADES 1941). Though this phenomenon has been known since the early days of genetics (MORGAN 1912), it still remains an enigma.

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SUMMARY

A localized genetic factor prevented any transmission of the carrier strand through the female gametophyte, while its transmission through the male was about 32%. This is at variance with the Mendelian principle of random segregation. RENNER-effect and polyspory have been ruled out; preferential segregation has not been ascertained. On the basis of embryological observation, megasporocyte selection is suggested as a possible mechanism responsible for the apparent discrepancy from the second Mendelian law. For a specific chromosome region, recombination in the presence of this factor is lower in the female than in the male ($P = 0.01$). The relation of megasporocyte selection to the crossing-over difference in the two sexes is not clarified by the experiments.

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